

TOPICS



Immunopathology

This document forms **Appendix 7** to **Trainee Handbook for Immunopathology** and should be read in conjunction with **Section 2** of the Handbook

The document was prepared in April 2009 by A/Prof David Fulcher, with contributions from Dr Richard Wong, Dr Melanie Wong and A/Prof William Sewell and was revised in March 2011.

1 Functions as a medical specialist in the laboratory

1.1. Knowledge and skills in immunobiology & pathogenesis of immunological disorders

1.1.1. Organisation of the immune system

- 1.1.1.1. Lymphoid tissues and organs: anatomy and function
- 1.1.1.2. Cells of relevance to the immune response, ontogeny, characteristics and functions
- 1.1.1.3. Molecules of relevance to the immune response: MHC, cytokines, chemokines, receptors, signalling pathways, vasoactive mediators, prostaglandins, leukotrienes
- 1.1.1.4. Genes of relevance to the immune response, including genes for above molecules and MHC, gene rearrangement in generation of antigen receptors
- 1.1.1.5. Structure and function relationships between the organs, tissues and cells that participate in immune responses
- 1.1.1.6. Cellular and molecular correlates of immune function
- 1.1.1.7. Comprehensive understanding of the ontogeny of the immune system and the relationship between the development of immune function to development of allergic diseases, autoimmunity, vasculitides and immunodeficiencies.

1.1.2. Immune mechanisms

- 1.1.2.1. Innate immunity:
 - 1.1.2.1.1. NK cells
 - 1.1.2.1.1.1. Cytokines relevant in NK cell development and function
 - 1.1.2.1.1.2. Activation and inhibition
 - 1.1.2.1.1.3. Cytotoxicity mechanisms
 - 1.1.2.1.2. Macrophages
 - 1.1.2.1.3. Granulocytes
 - 1.1.2.1.4. Receptors:
 - 1.1.2.1.4.1. Pattern recognition molecules (e.g. TLRs, MMR)
 - 1.1.2.1.4.2. Pathogen-associated molecular patterns;
 - 1.1.2.1.5. Proteins
 - 1.1.2.1.5.1. Complement
 - 1.1.2.1.5.2. Acute phase proteins
 - 1.1.2.1.5.3. Mannose-binding lectin
 - 1.1.2.1.5.4. Ficolins
 - 1.1.2.1.5.5. Anti-microbial peptides

1.2. Knowledge of immunologic disorders, and disorders relevant to the breadth of Immunopathological testing

1.2.1. Autoimmune diseases

- 1.2.1.1. Non-organ-specific autoimmune disorders (including SLE, RA, scleroderma, Sjögren's syndrome, MCTD)
- 1.2.1.2. Immune endocrinopathies
- 1.2.1.3. Autoimmune liver diseases
- 1.2.1.4. Coeliac disease
- 1.2.1.5. Inflammatory bowel diseases
- 1.2.1.6. Immunologic neurologic and neuromuscular diseases
- 1.2.1.7. Immunohaematologic diseases
- 1.2.1.8. Immunological ocular diseases
- 1.2.1.9. Vasculitic diseases
 - 1.2.1.9.1. Small, medium and large vessel vasculitides
 - 1.2.1.9.2. ANCA-positive vs negative vasculitides
 - 1.2.1.9.3. Pulmonary-renal syndromes
- 1.2.1.10. Immunodeficiency diseases (see subsection Y)
- 1.2.1.11. Allergic diseases
 - 1.2.1.11.1. Atopic disorders (allergic rhinitis/sinusitis/conjunctivitis, asthma, atopic dermatitis, food allergy)

- 1.2.1.11.2. Aspirin-sensitivity syndromes
- 1.2.1.11.3. Urticaria, angioedema
- 1.2.1.11.4. Mastocytosis
- 1.2.2. **Malignancies of the immune system (clinical presentation and diagnosis)**
 - 1.2.2.1. B cell and plasma cell neoplasms
 - 1.2.2.2. T cell neoplasms
 - 1.2.2.3. Monocyte/macrophage/DC/NK cell neoplasms

1.3. Knowledge of the principles, role and interpretation of diagnostic laboratory assays, including the ability to perform such assays where appropriate

- 1.3.1. **Polyclonal antisera**
 - 1.3.1.1. Explain the process of preparation of polyclonal antisera, including:
 - 1.3.1.1.1. Immunisation of animals
 - 1.3.1.1.2. Use of adjuvants
 - 1.3.1.1.3. Collection of blood
 - 1.3.1.1.4. Methods for purification of IgG
 - 1.3.1.1.5. Affinity purification (positive and negative)
 - 1.3.1.1.6. Testing of potency
- 1.3.2. **Monoclonal antibodies**
 - 1.3.2.1. Explain the process of preparation of monoclonal antibodies, including:
 - 1.3.2.1.1. Immunisation of animals
 - 1.3.2.1.2. Collection of cells and generation of hybridomas
 - 1.3.2.1.3. Selection and testing of clones
 - 1.3.2.1.4. Production of supernatant
 - 1.3.2.1.5. Methods for immunoglobulin purification
- 1.3.3. **Labelling of antibodies**
 - 1.3.3.1. Explain the process of labelling of polyclonal and monoclonal antibodies, including:
 - 1.3.3.1.1. Conjugation to FITC
 - 1.3.3.1.2. Conjugation to biotin
 - 1.3.3.1.3. Principles of biotin-avidin interaction
 - 1.3.3.1.4. Conjugation to protein fluorochromes, including tandem dyes
 - 1.3.3.2. Use of second stage reagents, including
 - 1.3.3.2.1. Anti-Ig reagents (animal-specific, poly-Ig-specific, IgG-specific, subclass specific)
 - 1.3.3.2.2. Streptavidin conjugates
- 1.3.4. **Immune-complex-based immunoassay**
 - 1.3.4.1. Explain the nature of antigen-antibody reactions in fluid phase, particularly with regard to:
 - 1.3.4.1.1. Nature of immune complexes
 - 1.3.4.1.2. Zones of Ab excess, equivalence and Ag excess
 - 1.3.4.2. Explain the principles of nephelometry and turbidimetry
 - 1.3.4.2.1. Light scatter characteristics of immune complexes
 - 1.3.4.2.2. Factors interfering with light scatter
 - 1.3.4.2.3. Antigen excess, and means for detection
 - 1.3.4.3. Discuss problems arising from antigen/Ab excess ("hook" effect, prozone)
- 1.3.5. **Enzyme-Linked ImmunoSorbent assay (ELISA)**
 - 1.3.5.1. Explain the principles of ELISA, including:
 - 1.3.5.1.1. Antibody detection, particularly detection of autoantibody
 - 1.3.5.1.2. Antigen detection (sandwich ELISA)
 - 1.3.5.1.3. Competitive ELISA
 - 1.3.5.2. Explain the use of enzyme-linked conjugates
 - 1.3.5.2.1. Procedures for using second-stage reagents
 - 1.3.5.2.2. List the commonly-used enzymes and their substrates
 - 1.3.5.3. Explain the principles of spectrophotometry

- 1.3.5.4. Explain the operation of laboratory equipment used in ELISA
 - 1.3.5.4.1. Plate washers
 - 1.3.5.4.2. OD readers
 - 1.3.5.4.3. Automated ELISA workstations
- 1.3.5.5. Explain the technical aspects of ELISA, including
 - 1.3.5.5.1. Appropriate buffers for each stage
 - 1.3.5.5.2. Methods to reduce non-specific binding
 - 1.3.5.5.3. Blocking reagents
 - 1.3.5.5.4. Detergents
 - 1.3.5.5.5. Choice of antibody reagents, including determining working dilutions
 - 1.3.5.5.6. Optimisation of assay conditions
 - 1.3.5.5.7. Explain the concept of optical density (OD)
 - 1.3.5.5.8. "Blank" versus "Zero" wells
- 1.3.5.6. Graph standard curves of ELISAs based on OD readouts and interpret sample results
- 1.3.5.7. List pitfalls of ELISA-based immunoassay
- 1.3.5.8. Explain the use of checkerboard titration in optimising assay conditions
- 1.3.6. **Radioimmunoassay (RIA)**
 - 1.3.6.1. List the characteristics of the three major particles of radioactivity
 - 1.3.6.1.1. Alpha
 - 1.3.6.1.2. Beta
 - 1.3.6.1.3. Gamma
 - 1.3.6.2. Define commonly-used units of radioactivity
 - 1.3.6.3. Explain the use of solid and liquid scintillators
 - 1.3.6.3.1. Distinguish between counts per minute (cpm) and disintegrations per minute (dpm)
 - 1.3.6.4. List radio-isotopes commonly used in the laboratory along with their specific characteristics
 - 1.3.6.4.1. Explain methods used to conjugate or incorporate these radio-isotopes into reagents used for immunoassay
 - 1.3.6.5. Define terms used in RIA, including
 - 1.3.6.5.1. "Binder", "ligand" and "analyte"
 - 1.3.6.5.2. "Blank" and "Zero" wells
 - 1.3.6.5.3. Scatchard plots and measurement of K
 - 1.3.6.6. Explain the principles behind RIA, including
 - 1.3.6.6.1. Physico-chemical separation of Bound versus Free
 - 1.3.6.6.2. Methods used for separation
 - 1.3.6.7. Explain the methodology behind specific types of RIA, including
 - 1.3.6.7.1. Classic RIA
 - 1.3.6.7.2. Immunoradiometric Assay (IRMA)
 - 1.3.6.7.3. Sandwich
 - 1.3.6.8. Graph standard curves of RIAs based on radioactivity readouts and interpret sample results
 - 1.3.6.8.1. Explain various methods for graphing standard curves
 - 1.3.6.8.2. Define the working range of an assay
- 1.3.7. **Agglutination**
 - 1.3.7.1. Explain the principles of the haemagglutination assay, including advantages and disadvantages compared with alternate methods
 - 1.3.7.1.1. Methods for conjugation of antigen to red blood cells
 - 1.3.7.1.2. Identification of endpoint
 - 1.3.7.2. Explain the principles of the particle agglutination assay, including advantages and disadvantages compared with alternate methods
- 1.3.8. **Luminex technology**
 - 1.3.8.1. Explain the principles of luminex technology in immunological assays, including advantages and disadvantages compared with alternate methods
 - 1.3.8.1.1. Autoantibody detection

- 1.3.8.1.2. Multiple cytokine analysis
- 1.3.9. **Western blot (WB)**
 - 1.3.9.1. Explains the principles of the WB assay
 - 1.3.9.1.1. Separation of proteins by molecular weight
 - 1.3.9.1.2. Blotting
 - 1.3.9.1.3. Incubation with serum
 - 1.3.9.1.4. Identification of bound antibody
 - 1.3.9.2. Interprets WB assays for the diagnosis of HIV infection (see HIV section)
- 1.3.10. **Miscellaneous reporter methods – chemiluminescence and fluorescent immunoassay**
 - 1.3.10.1. Explains the principles and application of
 - 1.3.10.1.1. Chemiluminescence assays
 - 1.3.10.1.2. Fluorescent immunoassay (FIA)
- 1.3.11. **Immunoprecipitation in gels**
 - 1.3.11.1. Explain the specific characteristics of the gels commonly used in immunochemistry laboratories
 - 1.3.11.2. Be able to prepare gels within the laboratory for specific purposes, including the use of templates and punching wells and troughs
 - 1.3.11.3. Explain the nature of antigen-antibody reactions in gels, particularly with regard to:
 - 1.3.11.3.1. Zones of Ab excess, equivalence and Ag excess (prozone effect)
 - 1.3.11.3.2. Principles of Ouchterlony testing
 - 1.3.11.3.3. Radial immunodiffusion assays, including end-point and fixed-time assays
 - 1.3.11.4. Recognise the pitfalls of gel precipitation assays, including
 - 1.3.11.4.1. Methodological issues, such as well overfilling/distortion, gel dessication
 - 1.3.11.4.2. Ag excess
 - 1.3.11.5. Interpret assays based on gel diffusion methods
 - 1.3.11.5.1. Fungal and avian precipitins
 - 1.3.11.5.2. Testing for anti-ENA antibodies, including identity, non-identity and partial identity
- 1.3.12. **Gel electrophoresis**
 - 1.3.12.1. Explain the physiology of the behaviour of proteins in a gel under the influence of an electric field, including the effects of
 - 1.3.12.1.1. Protein charge
 - 1.3.12.1.2. Buffer, especially pH
 - 1.3.12.1.3. Medium
 - 1.3.12.1.3.1. Agarose gel
 - 1.3.12.1.3.2. Nitrocellulose acetate
 - 1.3.12.1.4. Heat
 - 1.3.12.1.5. Endosmotic flow
 - 1.3.12.2. List the bands found in a standard and high-resolution electrophoretogram (EPG) of serum, including:
 - 1.3.12.2.1.1. The predominant proteins that comprise each band
 - 1.3.12.2.1.2. Their functions
 - 1.3.12.2.1.3. Their changes in states of health and disease
 - 1.3.12.3. Recognise the EPG patterns typical of other body fluids, including urine and CSF
 - 1.3.12.4. Identify patterns of changes in EPGs in health and disease states, including
 - 1.3.12.4.1. Paraproteins
 - 1.3.12.4.2. Hypogammaglobulinaemia
 - 1.3.12.4.3. Acute phase response
 - 1.3.12.4.4. Circulating immune complexes
 - 1.3.12.4.5. Nephrotic syndrome
 - 1.3.12.4.6. Liver disease
 - 1.3.12.4.7. Pregnancy
 - 1.3.12.4.8. Bisalbuminaemia
 - 1.3.12.4.9. Alpha-1-antitrypsin deficiency

- 1.3.12.4.10. Iron deficiency
- 1.3.12.4.11. Haemolysis
- 1.3.12.5. Explain the principles and be able to interpret immunoassays that utilise the migration of proteins within a gel, such as
 - 1.3.12.5.1. Counter-immunoelectrophoresis (CIEP), particularly as it applies to ENA detection
 - 1.3.12.5.2. Rocket electrophoresis (including fused rockets)
- 1.3.13. **Immunelectrophoresis (IEPG) and Immunofixation (IFE)**
 - 1.3.13.1. Explain the principles of serum IFE and IEPG for detection of paraproteinaemia
 - 1.3.13.2. Discuss advantages and disadvantages of serum IFE versus IEPG for detection of paraproteinaemia
 - 1.3.13.3. Interpret patterns of serum IFE and IEPG in health and disease, particularly in the detection of paraprotein-related disorders
 - 1.3.13.3.1. Monoclonal gammopathy of uncertain significance (MGUS)
 - 1.3.13.3.2. Multiple myeloma and plasmacytoma
 - 1.3.13.3.3. Waldenstrom's macroglobulinaemia
 - 1.3.13.3.4. Heavy chain disease
 - 1.3.13.3.5. Other B-cell lymphoproliferative disorders
 - 1.3.13.4. Explain the principles of CSF immunofixation
 - 1.3.13.5. Interpret patterns of CSF immunofixation, particularly for the detection of oligoclonal bands
 - 1.3.13.5.1. Calculate and interpret the CSF index
 - 1.3.13.5.2. List the differential diagnosis for the presence of oligoclonal bands in CSF
- 1.3.14. **Isoelectric focussing (IEF)**
 - 1.3.14.1. Explain the principles of IEF
 - 1.3.14.2. List applications of IEF
 - 1.3.14.2.1. Oligoclonal band detection
 - 1.3.14.2.2. Paraprotein detection
 - 1.3.14.2.3. Alpha-1-antitrypsin phenotyping
 - 1.3.14.3. Discuss advantages and disadvantages of IEF in comparison to other EPG methods
- 1.3.15. **Capillary zone electrophoresis (CZE)**
 - 1.3.15.1. Explain the principles of CZE
 - 1.3.15.2. List applications of CZE
 - 1.3.15.3. Discuss advantages and disadvantages of CZE in comparison to other EPG methods
 - 1.3.15.3.1. List differences in CZE patterns (ie specific protein detection) in comparison to gel EPG
 - 1.3.15.4. Interpret patterns of CZE and immunosubtraction in health and disease
 - 1.3.15.5. Explain the principles of immunosubtraction
 - 1.3.15.6. Discuss advantages and disadvantages of immunosubtraction in comparison to IFE and IEPG for detection of paraproteinaemia
- 1.3.16. **Cryoglobulinaemia**
 - 1.3.16.1. Explain the nature of cryoglobulins
 - 1.3.16.2. List the three major subtypes of cryoglobulins and the tests needed to differentiate them
 - 1.3.16.2.1. Interpret IFE/IEPG analysis of cryoprecipitate
 - 1.3.16.3. Explain and perform detection of cryoglobulins in the laboratory
 - 1.3.16.3.1. Explain the collection procedure
 - 1.3.16.3.2. Discuss the interpretation of a cryoglobulin assay, including verification, quantification and measurement of thermal amplitude
 - 1.3.16.4. Provide a differential diagnosis for a positive cryoglobulin result, including recommendation for further laboratory testing
 - 1.3.16.5. Explain the nature, detection and clinical implications of cryofibrinogen

 (Reference: Tedeschi A. Barate C. Minola E. Morra E. Cryoglobulinemia. *Blood Reviews*. 21(4):183-200, 2007 Jul.)

1.3.17. Complement

- 1.3.17.1. Explain the methodology for measurement of the complement pathways and their constituents, including:
 - 1.3.17.1.1. C3, C4, C1q
 - 1.3.17.1.2. CH50, CH100, AH50, AH100
 - 1.3.17.1.3. MBL pathway
- 1.3.17.2. Interpret abnormalities of complement tests in the clinical context
 - 1.3.17.2.1. List the causes of decrease or increase in C3, C4, C1q levels
 - 1.3.17.2.2. Detection of complement deficiency states, and strategies for further investigation
 - 1.3.17.2.3. Identifies changes brought about by autoantibodies targeting the complement system, including C3 and C4 nephritic factors
- 1.3.17.3. Explain the principles and interpretation of assays to assess the integrity of C1-inhibitor levels and function

1.3.18. Immunoglobulin levels and function

- 1.3.18.1. Interpret changes in immunoglobulin levels within the clinical context, including:
 - 1.3.18.1.1. Immunodeficiency states (see below)
 - 1.3.18.1.2. Disorders characterised by hypergammaglobulinaemia, including:
 - 1.3.18.1.2.1. Sjögren's syndrome
 - 1.3.18.1.2.2. Rheumatoid arthritis
 - 1.3.18.1.2.3. Chronic infection/inflammation

1.3.19. Light chain disorders

- 1.3.19.1. List clinical conditions associated with abnormal production of immunoglobulin light chains
 - 1.3.19.1.1. Myeloma (especially light chain) & plasmacytoma
 - 1.3.19.1.2. AL amyloidosis
 - 1.3.19.1.3. B-cell lymphoproliferative disorders
- 1.3.19.2. Explain the clinical and laboratory features of amyloidosis, including
 - 1.3.19.2.1. Specific amyloid subtypes
 - 1.3.19.2.1.1. AL amyloidosis
 - 1.3.19.2.1.2. AA amyloidosis
 - 1.3.19.2.1.3. Familial
 - 1.3.19.2.1.4. Other
 - 1.3.19.2.2. Identify amyloid deposition in tissues
 - 1.3.19.2.2.1. Congo red staining (DIF)
 - 1.3.19.2.2.2. Light chain staining (DIF)
 - 1.3.19.2.2.3. (Electron microscopy)
- 1.3.19.3. Explain the methodology for measurement of serum-free light chains (SFLC)
- 1.3.19.4. Interpret changes in SFLC within the clinical context

1.3.20. Miscellaneous serum proteins: C reactive protein, procalcitonin, alpha-1-antitrypsin

- 1.3.20.1. Interpret changes in C-reactive Protein (CRP) levels within the clinical context, including the ability to:
 - 1.3.20.1.1. Explain the structure, synthesis, biology and function of CRP
 - 1.3.20.1.2. List the causes of an elevated CRP and the indications for CRP measurement
 - 1.3.20.1.3. Discuss the role of high sensitivity CRP assays in cardiovascular disease
 - 1.3.20.1.4. Discuss the role of CRP in diagnosis and management of specific systemic inflammatory disorders
- 1.3.20.2. Interpret changes in Procalcitonin (PCT) levels within the clinical context
 - 1.3.20.2.1. Explain the structure, synthesis, biology and function of PCT
 - 1.3.20.2.2. Discuss the role of PCT in diagnosis and management of systemic infections
- 1.3.20.3. Interpret changes in alpha-1-antitrypsin (A1AT) levels within the clinical context, including the ability to:
 - 1.3.20.3.1. Recognise A1AT deficiency on EPG
 - 1.3.20.3.2. List common alleles of A1AT, and their clinical implications

1.3.20.3.3. Explain methods for phenotyping and genotyping

1.3.21. Specific antibodies, when assessing dynamic humoral immune response

1.3.21.1. Explain the methodology used to measure specific antibody responses in the assessment of dynamic humoral immune responses

1.3.21.2. Interpret responses to commonly used vaccines, including:

1.3.21.2.1. Pneumovax

1.3.21.2.2. Prevenar

1.3.21.2.3. Haemophilus influenzae type B (HIB)

1.3.21.2.4. Tetanus

1.3.21.2.5. Diphtheria

1.3.21.3. Discuss criteria for the diagnosis of specific/functional antibody deficiency and therapeutic implications

 *Jeurissen A. Moens L. Raes M. Wuyts G. Willebrords L. Sauer K. Proesmans M. Ceuppens JL. De Boeck K. Bossuyt X. Laboratory diagnosis of specific antibody deficiency to pneumococcal capsular polysaccharide antigens Clinical Chemistry. 53(3):505-10, 2007 Mar.*

 *Wernette CM. Frasch CE. Madore D. Carlone G. Goldblatt D. Plikaytis B. Benjamin W. Quataert SA. Hildreth S. Sikkema DJ. Kayhty H. Jonsdottir I. Nahm MH. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clinical & Diagnostic Laboratory Immunology. 10(4):514-9, 2003 Jul.*

1.3.22. Rheumatoid factor (RF)

1.3.22.1. Define the nature and specificity of RFs

1.3.22.2. List methods for RF detection, explaining the principles of each assay, and advantages and disadvantages

1.3.22.2.1. Nephelometry/turbidimetry

1.3.22.2.2. Particle agglutination

1.3.22.2.3. Haemagglutination

1.3.22.3. List causes of a positive RF

1.3.22.4. Interpret results for RF within the clinical context, including sensitivity and specificity

1.3.23. Indications for autoimmune serology testing

1.3.23.1. Advise clinicians on appropriate screening and confirmatory tests of autoimmune diseases based on clinical data

1.3.24. General techniques in autoimmune serology - explain principles, practice, application of techniques, troubleshooting, pros and cons

1.3.24.1. Explain the principles of fluorescence emission from various fluorochromes

1.3.24.2. Explain the principles of fluorescence and phase microscopy, including the use of specific filters

1.3.24.3. Operate a fluorescence microscope, including photography

1.3.24.4. Explain in detail the method for autoantibody detection using tissue substrates

1.3.24.4.1. Collection and preparation of tissue substrates

1.3.24.4.2. Embedding and snap-freezing

1.3.24.4.3. Operation of cryotome

1.3.24.4.4. Preparation and fixation of slides

1.3.24.4.5. Storage

1.3.24.5. Perform indirect immunofluorescence (IIF) with tissue substrates used for autoantibody detection

1.3.24.5.1. Perform end-point titration

1.3.24.5.2. Explain the use of checkerboard titration in optimising assay conditions

1.3.24.6. Discuss optimisation of IIF assays, including

1.3.24.6.1. Sample dilution and titration strategy

1.3.24.6.2. Choice of conjugate

1.3.24.6.3. Optimisation of conjugate

1.3.24.6.4. Checkerboard titration

1.3.25. Antinuclear antibodies – explain principles and relative merits of different substrates, interpret results

 *Reference: Muro Y. Antinuclear antibodies. Autoimmunity. 38(1):3-9, 2005 Feb.*

- 1.3.25.1. Discuss the advantages and disadvantages of alternative substrates for ANA detection, including
 - 1.3.25.1.1. HEp-2
 - 1.3.25.1.2. HEp-2000
 - 1.3.25.1.3. Solid tissue substrates
 - 1.3.25.1.4. ANA-ELISA
- 1.3.25.2. Interpret ANA patterns on HEp-2 and HEp-2000 substrates, including mixed patterns, considering the role of titration or fluorescence intensity in interpretation; for each pattern, list the related antigenic specificity(ies)
 - 1.3.25.2.1. Membranous/Rim
 - 1.3.25.2.2. Homogeneous
 - 1.3.25.2.3. Speckled
 - 1.3.25.2.3.1. Large speckled
 - 1.3.25.2.3.2. Coarse speckled
 - 1.3.25.2.3.3. Fine speckled
 - 1.3.25.2.3.4. Centromere
 - 1.3.25.2.3.5. Few (2-6) nuclear dots (Coilin p80)
 - 1.3.25.2.3.6. Multiple (5-10) nuclear dots (Sp-100)
 - 1.3.25.2.3.7. Scl-70 pattern
 - 1.3.25.2.4. Nucleolar
 - 1.3.25.2.4.1. Homogeneous (PM-Scl)
 - 1.3.25.2.4.2. Clumpy (Fibrillarin)
 - 1.3.25.2.4.3. Speckled (RNA polymerase)
 - 1.3.25.2.4.4. Speckled with mitotic dots (NOR90)
 - 1.3.25.2.5. Peripheral nuclear staining
 - 1.3.25.2.5.1. Nuclear pore complex (gp210, Nup62, Tpr, Nup153)
 - 1.3.25.2.5.2. Lamins
 - 1.3.25.2.6. MSA patterns
 - 1.3.25.2.6.1. Mitotic spindle apparatus (MSA-1)
 - 1.3.25.2.6.2. Mitotic spindle pole (NuMA)
 - 1.3.25.2.6.3. Midbody (MSA-2)
 - 1.3.25.2.6.4. Granules around metaphase plate (MSA-3)
 - 1.3.25.2.7. Cytoplasmic:
 - 1.3.25.2.7.1. Jo-1
 - 1.3.25.2.7.2. Ribosomal
 - 1.3.25.2.7.3. Mitochondrial
 - 1.3.25.2.7.4. Golgi
 - 1.3.25.2.7.5. Lysosomal
 - 1.3.25.2.7.6. Filamentous
 - 1.3.25.2.7.7. Actin
 - 1.3.25.2.7.8. Signal Recognition Peptide
 - 1.3.25.2.7.9. 1.3.25.2.7.9. Endoplasmic reticulum
 - 1.3.25.2.7.10. Centriole / centrosome
- 1.3.25.3. Interpret positive ANAs within the clinical context, including sensitivity and specificity, considering:
 - 1.3.25.3.1. Role of ANA titre
 - 1.3.25.3.2. Influence of age
 - 1.3.25.3.3. Relevance of pattern
- 1.3.25.4. Recommend further investigation as appropriate

1.3.26. Extractable Nuclear Antigens (ENA) – explain principles and relative merits of different assays, interpret results

1.3.26.1. List assays for ENA detection, explaining the principles behind the assays, and their relative merits, including

- 1.3.26.1.1. ELISA
- 1.3.26.1.2. Ouchterlony and Couter-immunoelectrophoresis
- 1.3.26.1.3. Line immunoassay

1.3.26.2. Explain the principles of ENA assays

1.3.26.3. Interpret assays for ENA detection

- 1.3.26.3.1. Interpret lines of identity, non-identity and partial identity
- 1.3.26.3.2. LIA

1.3.26.4. Interpret positive ENAs within the clinical context, including sensitivity and specificity

- 1.3.26.4.1. SSA/Ro
- 1.3.26.4.2. TRIM-21 (Ro52)
- 1.3.26.4.3. SSB/La
- 1.3.26.4.4. Sm
- 1.3.26.4.5. (U1)-RNP
- 1.3.26.4.6. Jo-1
- 1.3.26.4.7. Scl-70 (Topoisomerase I)
- 1.3.26.4.8. PM-Scl
- 1.3.26.4.9. Ribosomal P
- 1.3.26.4.10. Fibrillarin

1.3.27. Double-stranded DNA (dsDNA) and related autoantibodies - explain principles and relative merits of different assays, interpret results

1.3.27.1. List assays for anti-dsDNA Abs, explaining the principles behind the assays, and their relative merits, including

- 1.3.27.1.1. Farr
- 1.3.27.1.2. Crithidia
- 1.3.27.1.3. ELISA

1.3.27.2. Interpret positive anti-dsDNA results within the clinical context, including sensitivity and specificity

1.3.27.3. List assays for anti-histone Abs, explaining the principles behind the assays, and their relative merits, including

- 1.3.27.3.1. IIF
- 1.3.27.3.2. ELISA

1.3.27.4. Interpret positive anti-histone Abs results within the clinical context, including sensitivity and specificity

1.3.27.5. Discuss the role of anti-nucleosome ELISA testing

- 1.3.27.5.1. Compare assay performance with tests for anti-dsDNA Abs
- 1.3.27.5.2. Interpret results within the clinical context, including sensitivity and specificity

1.3.28. Antineutrophil cytoplasmic antibody (ANCA) - explain principles and relative merits of different assays, interpret results

1.3.28.1. List assays for ANCA, explaining the principles behind the assays, and their relative merits, including

- 1.3.28.1.1. ANCA by IIF
- 1.3.28.1.2. ELISA

1.3.28.2. Detect ANCA by IIF on neutrophil substrate

1.3.28.3. Interpret positive ANCA results within the clinical context, including sensitivity and specificity.

1.3.29. Autoimmune liver disease (AILD) – explain the clinical and laboratory features, list the relevant antibodies and explain methods for detection and their relative merits.

- 📖 *Ref: Vergani D. Alvarez F. Bianchi FB. Cancado EL. Mackay IR. Manns MP. Nishioka M. Penner E. International Autoimmune Hepatitis Group. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. Journal of Hepatology. 41(4):677-83, 2004 Oct.*

📖 *Francesca Meda, Massimo Zuin, Pietro Invernizzi, Diego Vergani & Carlo Selmi. Serum autoantibodies: A road map for the clinical hepatologist. Autoimmunity: 41(1): 27–34, 2008*

- 1.3.29.1. Explain the clinical and laboratory features of the three main categories of autoimmune liver disease, namely:
 - 1.3.29.1.1. Autoimmune (chronic active) hepatitis (AIH)
 - 1.3.29.1.2. Primary biliary cirrhosis (PBC)
 - 1.3.29.1.3. Primary sclerosing cholangitis (PSC)
- 1.3.29.2. List the autoantibodies relevant to AILD, and discuss their diagnostic relevance in the categorisation of AILD, including
 - 1.3.29.2.1. Smooth Muscle Antibodies (SMA)
 - 1.3.29.2.2. Liver-kidney microsomal antibodies (LKM)
 - 1.3.29.2.3. Mitochondrial Antibodies (AMA)
 - 1.3.29.2.4. ANCA (see above)
 - 1.3.29.2.5. ANA (see above)
 - 1.3.29.2.6. Liver soluble liver antigen (anti-SLA)/anti liver pancreas antibodies (anti-LP)
 - 1.3.29.2.7. Liver cytosol antibodies (anti-LC1)
 - 1.3.29.2.8. Asialoglycoprotein receptor antibodies (anti-ASGPR)
- 1.3.29.3. Detect SMA by IIF of 'composite block' tissue substrates
 - 1.3.29.3.1. Differentiate SMA-V, -VG and -VGT patterns and explain
 - 1.3.29.3.1.1. Autoantigens associated with each pattern
 - 1.3.29.3.1.2. Diagnostic implications of each pattern
 - 1.3.29.3.2. List other methods for detection of SMA
 - 1.3.29.3.3. Interpret positive SMA results within the clinical context, including sensitivity and specificity
- 1.3.29.4. Detect LKM by IIF on 'composite block' tissue substrates
 - 1.3.29.4.1. List autoantigens associated with LKM1, 2 & 3
 - 1.3.29.4.2. Interpret positive LKM results within the clinical context, including sensitivity and specificity
- 1.3.29.5. Detect AMA by IIF on 'composite block' tissue substrates
 - 1.3.29.5.1. Explain the masking of GPC Abs
 - 1.3.29.5.2. Explain the subtypes of AMA
 - 1.3.29.5.2.1. Explain the nature of the autoantigen associated with M2-AMA, and its function
 - 1.3.29.5.3. List other methods for detection of AMA, including
 - 1.3.29.5.3.1. ELISA
 - 1.3.29.5.3.2. LIA
 - 1.3.29.5.4. Interpret positive AMA results within the clinical context, including sensitivity and specificity
- 1.3.30. **Antiphospholipid antibodies – explain principles, practical application, relative merits of assays for these antibodies**
 - 1.3.30.1. List autoantibodies associated with antiphospholipid syndrome (aPLS), explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.30.1.1. Anticardiolipin Ab
 - 1.3.30.1.2. Lupus anticoagulant
 - 1.3.30.1.3. β -2-glycoprotein-I Ab
 - 1.3.30.2. Apply the results of these autoantibody tests, including sensitivity and specificity, to the diagnostic criteria for aPLS in the clinical context
 - 1.3.30.2.1. Discuss relevance of persistent versus transient antiphospholipid antibodies
- 1.3.31. **Coeliac disease – explain immunopathogenesis, list relevant antibodies and explain methods for detection and their relative merits**
 - 1.3.31.1. Explain the immunopathogenesis of coeliac disease
 - 1.3.31.2. List autoantibodies associated with coeliac disease, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.31.2.1. Endomysial Ab (EMA)
 - 1.3.31.2.2. Tissue transglutaminase Ab (TTG)

- 1.3.31.2.3. Gliadin IgG & IgA
 - 1.3.31.2.3.1. Deamidated gliadin assays
- 1.3.31.2.4. Actin IgA (AAAA)
- 1.3.31.2.5. Reticulin
- 1.3.31.3. Explain the use and diagnostic value of isotype-specific reagents
- 1.3.31.4. Detect EMA by IIF on oesophagus substrate
- 1.3.31.5. Interpret positive results within the clinical context, including sensitivity and specificity
 - 1.3.31.5.1. Including the association with IgA deficiency and the effect of this on test interpretation
- 1.3.32. **Autoantibodies in endocrine diseases - list relevant antibodies and explain methods for detection and their relative merits for Type 1 diabetes, autoimmune thyroid diseases, Addison's disease, pernicious anaemia/atrophic gastritis**
 - 1.3.32.1. Type I diabetes (T1DM)
 - 1.3.32.1.1. List autoantibodies associated with type I diabetes, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.32.1.1.1. Islet cell antibodies (ICA)
 - 1.3.32.1.1.2. Insulin antibodies
 - 1.3.32.1.1.3. Glutamic acid decarboxylase (GAD65) antibodies
 - 1.3.32.1.1.4. IA-2 (ICA512) antibodies
 - 1.3.32.1.2. Explain the use of these results in predicting onset of T1DM
 - 1.3.32.1.3. Detect ICA by IIF on pancreas substrate
 - 1.3.32.2. Autoimmune thyroid diseases
 - 1.3.32.2.1. List autoantibodies associated with autoimmune thyroid diseases, explaining the principles behind the assays, and their relative diagnostic merits for Hashimoto's and Grave's disease, including
 - 1.3.32.2.1.1. Thyroid peroxidase (microsomal; TPO) antibodies
 - 1.3.32.2.1.2. Thyroglobulin (TG) antibodies
 - 1.3.32.2.1.3. Thyroid stimulating immunoglobulin
 - 1.3.32.2.1.4. Thyroid receptor blocking antibodies
 - 1.3.32.2.1.5. Thyroid stimulating hormone (TSH) antibodies
 - 1.3.32.3. Addison's disease
 - 1.3.32.3.1. List autoantibodies associated with Addison's disease, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.32.3.1.1. Anti-adrenal antibody (IIF)
 - 1.3.32.3.2. Detect anti-adrenal antibody by IIF on adrenal cortex substrate
 - 1.3.32.4. Pernicious anaemia/atrophic gastritis
 - 1.3.32.4.1. List autoantibodies associated with pernicious anaemia/atrophic gastritis, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.32.4.1.1. Gastric parietal cell (GPC)
 - 1.3.32.4.1.2. Intrinsic factor
 - 1.3.32.4.2. List the autoantigen for GPC Ab
 - 1.3.32.4.2.1. H⁺/K⁺-ATPase
 - 1.3.32.4.3. Detect GPC Ab by IIF on 'composite block' tissue substrates
- 1.3.33. **Autoantibodies in autoimmune skin disorders - list autoantigens associated with these skin disorders, explain principles behind assays, their relative diagnostic merits, interpret results in clinical context**
 - 1.3.33.1. List autoantigens associated with the bullous skin disorders, along with their antigenic specificity, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.33.1.1. Intercellular cement substance Abs (ICSA)
 - 1.3.33.1.1.1. Desmoglein-1 & 3
 - 1.3.33.1.1.2. Desmocollins
 - 1.3.33.1.1.3. Plakin family
 - 1.3.33.1.2. Skin basement membrane Abs

- 1.3.33.1.2.1. BPAG-1 (230)
- 1.3.33.1.2.2. BPAG-2 (180)
- 1.3.33.1.2.3. Laminin-5
- 1.3.33.1.2.4. Collagen-VII
- 1.3.33.2. Detect ICSPA and SBMA by IIF on primate oesophagus substrate
 - 1.3.33.2.1. Differentiate pemphigus foliaceus from vulgaris using IIF on guinea-pig oesophagus
 - 1.3.33.2.2. Differentiate pemphigus from paraneoplastic pemphigus based on rat bladder staining
 - 1.3.33.2.3. Differentiate pemphigoid subtypes based on split skin substrate
- 1.3.33.3. Interpret positive results within the clinical context, including sensitivity and specificity
- 1.3.34. **Autoantibodies in neurological diseases**
 - 📖 *Dropcho, EJ. Update on paraneoplastic syndromes. Curr Opin Neurol 2005;18:331-336.*
 - 1.3.34.1. Myasthenia gravis
 - 1.3.34.1.1. Explain the immunopathogenesis of myasthenia gravis
 - 1.3.34.1.2. List autoantibodies associated with myasthenia gravis, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.34.1.2.1. -acetylcholine receptor Abs
 - 1.3.34.1.2.2. Skeletal muscle antibodies
 - 1.3.34.1.2.3. MuSK
 - 1.3.34.1.3. Interpret positive results within the clinical context, including sensitivity and specificity
 - 1.3.34.2. Paraneoplastic neuronal autoantibodies
 - 1.3.34.2.1. List autoantibodies associated with paraneoplastic neuronal syndromes, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.34.2.1.1. ANNA-1/Hu
 - 1.3.34.2.1.2. ANNA-2/Ri
 - 1.3.34.2.1.3. PCA-1/Yo
 - 1.3.34.2.2. Detect neuronal autoantibodies by IIF on primate cerebellum and intestine substrates
 - 1.3.34.2.3. Interpret positive results within the clinical context, including sensitivity and specificity
 - 1.3.34.3. Autoantibodies in peripheral neuropathy
 - 📖 *Willison HJ & Yuki N. Peripheral neuropathies and anti-glycolipid antibodies. Brain 125:2591-2625, 2002*
 - 1.3.34.3.1. Myelin-associated glycoprotein
 - 1.3.34.3.2. Ganglioside antibodies
- 1.3.35. **Autoantibodies in inflammatory bowel disease**
 - 1.3.35.1. List assays for the differentiation of Crohn's disease from ulcerative colitis, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.35.1.1. Anti-Saccharomyces cerevisiae antibody (ASCA)
 - 1.3.35.1.2. pANCA (see elsewhere)
 - 1.3.35.2. Interpret positive results within the clinical context, including sensitivity and specificity
- 1.3.36. **Autoantibodies associated with miscellaneous rheumatic conditions**
 - 1.3.36.1. List assays for the diagnosis of rheumatoid arthritis, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.36.1.1. Cyclic citrullinated peptide (CCP)
 - 1.3.36.1.2. RF (see elsewhere)
 - 1.3.36.2. List assays for the diagnosis of Sjogren's syndrome, explaining the principles behind the assays, and their relative diagnostic merits, including
 - 1.3.36.2.1. Salivary duct Abs
 - 1.3.36.2.2. SSA, SSB (see elsewhere)
- 1.3.37. **General techniques in flow cytometry**
 - 1.3.37.1. Explain the principles of operation of the flow cytometer

- 1.3.37.1.1. Fluidics and laminar flow
- 1.3.37.1.2. Detection of transmitted and scattered light
- 1.3.37.1.3. Choice of lasers and filters
- 1.3.37.1.4. Dual and triple-laser platforms
- 1.3.37.1.5. Photomultiplier tube voltage adjustment
- 1.3.37.1.6. Absorption and emission spectra of fluorochromes
- 1.3.37.1.7. Colour compensation (multiparameter)
- 1.3.37.1.8. Scatter characteristics of blood cells (white cells, red cells, platelets)
- 1.3.37.1.9. Data storage
- 1.3.37.1.10. Gating strategies
- 1.3.37.2. Be able to label cells for FCM, including:
 - 1.3.37.2.1. Ficoll-hypaque separation, cell counting and resuspension
 - 1.3.37.2.2. Whole blood lysis techniques
 - 1.3.37.2.3. Choice of staining strategies, considering:
 - 1.3.37.2.3.1. Species of origin of staining antibodies
 - 1.3.37.2.3.2. Second stage reagents
 - 1.3.37.2.3.3. Use of blocking techniques
 - 1.3.37.2.4. Use of isotype controls
- 1.3.37.3. Be able to analyse and interpret FCM data
 - 1.3.37.3.1. Histogram, dot-plot, contour analysis
 - 1.3.37.3.2. Interpretation of statistics
 - 1.3.37.3.3. Determination of positive versus negative populations
 - 1.3.37.3.4. Measurement of fluorescence intensity
- 1.3.37.4. Be able to identify blood cell populations based on scatter characteristics and CD45 expression.
- 1.3.37.5. List the methods for verifying the stability of the flow cytometer performance
 - 1.3.37.5.1. Critical performance parameters
 - 1.3.37.5.2. Labelled beads
 - 1.3.37.5.3. Normal control blood samples
- 1.3.38. **Lymphocyte enumeration**
 - 1.3.38.1. Name CD antigens useful in the identification of the major cellular constituents of blood, particularly B, T and NK cells
 - 1.3.38.1.1. Use of both CD45 and light scatter gating
 - 1.3.38.2. Interpret FCM data from stains of various blood cell populations, particularly lymphocytes
 - 1.3.38.3. Calculate absolute B, T and NK lymphocyte subset counts from the percentage data and absolute lymphocyte count.
 - 1.3.38.4. Explain the use of single-platform methods for quantification of absolute lymphocyte subset counts
 - 1.3.38.5. Explain and apply quality-control procedures in the measurement of lymphocyte subsets
 - 1.3.38.5.1. Calculation of lymphosum and acceptable limits
 - 1.3.38.5.2. Verification of lymphocyte scatter gates
 - 1.3.38.5.3. Use of external quality controls reagents
 - 1.3.38.5.4. QAP programs
 - 1.3.38.6. Explain the use of lymphocyte proportion and count in the diagnosis and management of patients with immunodeficiency disorders (see Immunodeficiency, HIV)
- 1.3.39. **Immunophenotyping of B-cell lymphoproliferative disorders**
 - 1.3.39.1. Name CD antigens specific for, and associated with, the B-cell lineage, including variation during development, and state the overlap of expression with other cell lineages
 - 1.3.39.2. Recognise patterns of antigen expression characteristic of B-lineage lymphoproliferative disease
 - 1.3.39.3. Recognise patterns of light chain expression implying clonal B-cell expansion

- 1.3.39.4. Identify phenotypic characteristics of distinct B-cell clonal disorders, including
 - 1.3.39.4.1. B-lineage ALL
 - 1.3.39.4.2. Burkitt's lymphoma
 - 1.3.39.4.3. B-cell chronic lymphocytic leukaemia (B-CLL)
 - 1.3.39.4.4. Mantle cell lymphoma (MCL)
 - 1.3.39.4.5. Follicular centre cell lymphoma (FCCL)
 - 1.3.39.4.6. Hairy cell leukaemia (HCL)
 - 1.3.39.4.7. Plasma cell dyscrasias
- 1.3.39.5. List prognostic markers in B-CLL detectable by flow cytometry
- 1.3.40. **Immunophenotyping of T-cell lymphoproliferative disorders**
 - 1.3.40.1. Name CD antigens specific for, and associated with, the T-cell lineage, and state the overlap of expression with other cell lineages
 - 1.3.40.2. Recognise altered patterns of antigen expression suggestive of clonal T-cell expansion
 - 1.3.40.3. Identify the phenotypic differences in the three stages of T-ALL / T-lymphoblastic leukaemia
- 1.3.41. **Immunophenotyping of NK-cell lymphoproliferative disorders**
 - 1.3.41.1. Be able to name CD antigens associated with the NK-cell lineage, and state the overlap of expression with other cell lineages
 - 1.3.41.2. Identify phenotypic and clinical characteristics of NK-LGL disorders
- 1.3.42. **Detection of mast cells**
 - 1.3.42.1. List cell surface markers used for detection of mast cells, and interpret immunophenotyping based on these markers
- 1.3.43. **Indications for HLA typing**
 - 1.3.43.1. Advise clinicians on appropriate indications for tissue typing based on clinical data
- 1.3.44. **HLA typing methodologies**
 - 1.3.44.1. 1.3.44.1. Explain the structure of the HLA system (see Basic Immunobiology)
 - 1.3.44.2. Explain the procedures used in HLA-typing, including
 - 1.3.44.2.1. Lymphocytotoxicity
 - 1.3.44.2.2. Flow cytometry
 - 1.3.44.2.3. Molecular methods
 - 1.3.44.2.3.1. PCR-SSO
 - 1.3.44.2.3.2. PCR-SSP
 - 1.3.44.2.3.3. SBT
 - 1.3.44.2.4. Mixed lymphocyte culture (MLC) / mixed lymphocyte reaction (MLR)
 - 1.3.44.2.5. HTLp, CTLp assays
 - 1.3.44.3. Interprets HLA pedigrees based on HLA typing within a family
 - 1.3.44.4. Advises on selection of donors for renal and bone marrow transplantation based on HLA pedigrees
- 1.3.45. **HLA in Immunological disease**
 - 1.3.45.1. List methods for detecting HLA-B27, explaining the principles behind the assays, and their clinical implications, including
 - 1.3.45.1.1. Flow cytometry
 - 1.3.45.1.1.1. Cross-reactivities and analysis strategies
 - 1.3.45.1.2. Lymphocytotoxicity
 - 1.3.45.1.3. Particle agglutination
 - 1.3.45.1.4. Immunomagnetic separation ELISA
 - 1.3.45.1.5. Molecular methods
 - 1.3.45.1.5.1. PCR-SSP
 - 1.3.45.1.5.2. PCR-SSO
 - 1.3.45.1.5.3. Sequencing
 - 1.3.45.1.5.4. FRET
 - 1.3.45.2. Explain the application of specific HLA typing in the diagnosis of
 - 1.3.45.2.1. Coeliac disease
 - 1.3.45.2.2. Drug sensitivity

1.3.45.2.2.1. Abacavir (HLA-B*5701)

1.3.45.2.2.2. Carbamazepine (HLA-B*1505)

1.3.46. Indications for allergy testing

📖 *Hamilton RG. Franklin Adkinson N Jr. In vitro assays for the diagnosis of IgE-mediated disorders. Journal of Allergy & Clinical Immunology. 114(2):213-25; quiz 226, 2004 Aug.*

1.3.46.1. Advise clinicians on appropriate screening and confirmatory tests of allergic disease based on clinical data

1.3.47. Total IgE measurement

1.3.47.1. List assays for the measurement of total IgE, explaining the principles behind the assays, and their relative advantages/disadvantages, including

1.3.47.1.1. IRMA

1.3.47.1.2. UniCAP

1.3.47.2. List the indications for measurement of total IgE

1.3.47.3. Interpret positive results within the clinical context, including sensitivity and specificity

1.3.48. Specific IgE measurement

1.3.48.1. List assays for the measurement of specific IgE, explaining the principles behind the assays, and their relative advantages/disadvantages, including

1.3.48.1.1. RAST

1.3.48.1.2. ImmunoCAP

1.3.48.1.3. Liquid allergen-based assays

1.3.48.2. List the indications for measurement of specific IgE

1.3.48.2.1. Contrast with skin prick testing

1.3.48.3. Interpret positive results within the clinical context, including sensitivity and specificity

1.3.48.4. Explain the principles of RAST inhibition, and list the applications

1.3.49. Mast cell and eosinophil products

1.3.49.1. Describe the structure and function of tryptase

1.3.49.2. Explain the biology of tryptase release in allergic reactions

1.3.49.3. List assays for the measurement of tryptase, explaining the principles behind the assays, and their clinical indications

1.3.49.4. Interpret tryptase results within the clinical context, including sensitivity and specificity for diagnosis of

1.3.49.4.1. Mastocytosis

1.3.49.4.2. Anaphylaxis

1.3.49.5. Explain the assay for the measurement of Eosinophil Cationic Protein (ECP), explaining the principles behind the assay, and their clinical indications

1.3.49.6. Interpret ECP results in the management of asthma

1.3.50. Investigation and interpretation of tests in patients with suspected immunodeficiency

📖 *Notarangelo L. Casanova JL. Conley ME. Chapel H. Fischer A. Puck J. Roifman C. Seger R. Geha RS. International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee Meeting in Budapest, 2005. Journal of Allergy & Clinical Immunology. 117(4):883-96, 2006 Apr.*

1.3.50.1. Advise clinicians on appropriate screening tests of immunodeficiency based on clinical data

1.3.50.2. Interpret tests of lymphocyte subset proportion, number and function

1.3.50.3. Advise on more specific testing available for immunodeficiency diagnosis

1.3.50.3.1. Western blotting

1.3.50.3.2. Flow cytometry assays

1.3.50.3.3. Genetic tests

1.3.51. B-cell immunodeficiency disorders

1.3.51.1. Interpret tests relevant to the assessment of B-cell immunodeficiency disorders, including lymphocyte subsets, Ig levels, dynamic Ab responses

1.3.51.1.1. Explain differences in normal ranges in paediatrics versus adults

1.3.51.2. Discuss the laboratory, clinical, genetic and pathophysiological aspects of B-cell immunodeficiency disorders, including:

- 1.3.51.2.1. Common variable immunodeficiencies (CVID), including
 - 1.3.51.2.1.1. TACI mutations
 - 1.3.51.2.1.2. Deficiency of other major proteins that cause the CVID phenotype (CD19, CD81, ICOS, BAFF-R etc)
- 1.3.51.2.2. IgA deficiency
- 1.3.51.2.3. X-linked agammaglobulinaemia (Bruton's)
- 1.3.51.2.4. IgG subclass deficiency
- 1.3.51.2.5. Specific antibody deficiency
- 1.3.51.2.6. Hyper-IgM syndromes ("Class-switch recombination disorders")
 - 1.3.51.2.6.1. CD40L deficiency
 - 1.3.51.2.6.2. CD40 deficiency
 - 1.3.51.2.6.3. AID deficiency
 - 1.3.51.2.6.4. UNG deficiency
 - 1.3.51.2.6.5. NEMO deficiency
- 1.3.51.2.7. X-linked lymphoproliferative disease (Duncan's)
- 1.3.51.3. Explain the process of B-cell memory immunophenotyping and its diagnostic and management implications for CVID
- 1.3.52. T-cell immunodeficiency disorders**
 - 1.3.52.1. Interpret lymphocyte subset changes in T-cell immunodeficiency disorders
 - 1.3.52.1.1. Explain differences in normal ranges in paediatrics versus adults
 - 1.3.52.2. Explain and interpret tests of T-cell function, including
 - 1.3.52.2.1. Mitogen blastogenesis
 - 1.3.52.2.1.1. ³H-thymidine
 - 1.3.52.2.1.2. Carboxyfluorescein succinimidyl ester (CFSE)
 - 1.3.52.2.2. Cytotoxic T-lymphocyte (CTL) function assays
 - 1.3.52.2.3. Tetramer staining
 - 1.3.52.2.4. γ -interferon release assays
 - 1.3.52.2.4.1. Quantiferon (Mycobacteria tuberculosis)
 - 1.3.52.3. Discuss the laboratory, clinical, genetic and pathophysiological aspects of T-cell deficiency disorders, including:
 - 1.3.52.3.1. Severe combined immunodeficiency
 - 1.3.52.3.1.1. Gamma common chain deficiency
 - 1.3.52.3.1.2. Jak-3 deficiency
 - 1.3.52.3.1.3. IL-7R α deficiency
 - 1.3.52.3.1.4. Adenosine deaminase (ADA) deficiency
 - 1.3.52.3.1.5. Purine nucleotide phosphorylase (PNP) deficiency
 - 1.3.52.3.1.6. Zap-70 deficiency
 - 1.3.52.3.1.7. CD3 deficiencies
 - 1.3.52.3.1.8. CD45 deficiency
 - 1.3.52.3.1.9. Rag-1 & 2 deficiency
 - 1.3.52.3.1.10. Bare lymphocyte syndrome types I & II
 - 1.3.52.3.2. Omenn's syndrome
 - 1.3.52.3.3. Myeloid or reticular dysgenesis
 - 1.3.52.3.4. Hyper-IgE syndrome
 - 1.3.52.3.5. Chronic mucocutaneous candidiasis
- 1.3.53. Complement disorders**
 - 1.3.53.1. Interpret tests relevant to the assessment of complement disorders
 - 1.3.53.2. Discuss the laboratory, clinical, genetic and pathophysiological aspects of complement disorders, including
 - 1.3.53.2.1. Defects of classical pathway complement components
 - 1.3.53.2.2. Defects of alternate pathway complement components
 - 1.3.53.2.3. Defects of mannose binding lectin
 - 1.3.53.2.4. Defects of common pathway complement components
 - 1.3.53.2.5. Defects of complement inhibitors

- 1.3.53.2.5.1. C1 inhibitor
- 1.3.53.2.5.2. Factor H & I
- 1.3.53.2.5.3. Paroxysmal nocturnal haemoglobinuria (PNH)
- 1.3.53.2.6. C3 and C4 nephritic factors
- 1.3.54. Neutrophil disorders**
 - 1.3.54.1. Discuss the laboratory, clinical, genetic and pathophysiological aspects of neutrophil disorders, including
 - 1.3.54.1.1. Chronic granulomatous disease
 - 1.3.54.1.2. Leukocyte adhesion disorders (LAD) types I & II
 - 1.3.54.1.3. Chediak-Higashi Syndrome
 - 1.3.54.1.4. Glucose-6-phosphate dehydrogenase deficiency
 - 1.3.54.1.5. Myeloperoxidase deficiency
 - 1.3.54.2. Interpret tests of neutrophil function, including
 - 1.3.54.2.1. Nitroblue tetrazolium (NBT) test
 - 1.3.54.2.2. Dihydrorhodamine reductase (DHR) test
 - 1.3.54.2.3. Chemiluminescence
 - 1.3.54.2.4. Neutrophil chemotaxis
- 1.3.55. NK cell disorders**
 - 1.3.55.1. Discuss the laboratory, clinical, genetic and pathophysiological aspects of NK cell disorders, including
 - 1.3.55.1.1. Familial haemophagocytic lymphohistiocytosis (FHL), including
 - 1.3.55.1.1.1. Perforin
 - 1.3.55.1.1.2. Munc 13-4 (UNC13D)
 - 1.3.55.1.1.3. Syntaxin 11
 - 1.3.55.1.2. Infection-associated haemophagocytic syndrome
 - 1.3.55.2. Explain and interpret tests of NK-cell function
- 1.3.56. Immunodeficiencies with other major defects**
 - 1.3.56.1. Discuss the laboratory, clinical, genetic and pathophysiological aspects of the following miscellaneous immunodeficiency disorders, including
 - 1.3.56.1.1. Di George syndrome
 - 1.3.56.1.2. Wiskott-Aldrich Syndrome
 - 1.3.56.1.3. Ataxia Telangiectasia
- 1.3.57. Miscellaneous immunodeficiency disorders**
 - 1.3.57.1. Discuss the laboratory, clinical, genetic and pathophysiological aspects of innate immunoreceptor/signalling immunodeficiency disorders, including
 - 1.3.57.1.1. Irak-4 deficiency
 - 1.3.57.1.2. TLR3 deficiency
 - 1.3.57.1.3. UNC93B deficiency
 - 1.3.57.2. Discuss the laboratory, clinical, genetic and pathophysiological aspects of APECED (autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy)
 - 1.3.57.3. Discuss the laboratory, clinical, genetic and pathophysiological aspects of IPEX (Immunodeficiency, polyendocrinopathy, enteropathy, X-linked)
 - 1.3.57.4. Discuss the laboratory, clinical, genetic and pathophysiological aspects of ALPS (autoimmune lymphoproliferative syndrome)
 - 1.3.57.5. Discuss the laboratory, clinical, genetic and pathophysiological aspects of defects in the IFN γ -IL-12 axis (Mendelian susceptibility to Mycobacterial Disease)
- 1.3.58. Diagnosis of HIV infection**
 - 1.3.58.1. Advise clinicians on appropriate screening and confirmatory tests of HIV infection based on clinical data
 - 1.3.58.2. List assays for the diagnosis of HIV-1 and HIV-2 infection, explaining the principles behind the assays, and their relative advantages/disadvantages, including
 - 1.3.58.2.1. Anti-HIV ELISA
 - 1.3.58.2.2. p24 antigen
 - 1.3.58.2.3. Ag-Ab combination ELISAs

1.3.58.2.4. WB

1.3.58.2.4.1. Explain and interpret the various indeterminate patterns and their implications

1.3.58.2.5. Proviral DNA

1.3.58.2.6. HIV culture

1.3.58.2.7. HIV viral load

1.3.58.3. Advise clinicians on the indications for each respective test

1.3.58.4. Interpret positive results within the clinical context, including sensitivity and specificity, and advise on further investigation

1.3.59. Monitoring of HIV infection

1.3.59.1. List assays for the measurement of HIV viral load (HIV-VL), explaining the principles behind the assays, and their relative advantages/disadvantages, including

1.3.59.1.1. RT-PCR

1.3.59.1.2. (Branched chain DNA)

1.3.59.2. Advise clinicians on the implications of HIV-VL results, and their impact on management

1.3.59.3. Explain the use of CD4 proportion and number in the management of HIV infection

1.4. General laboratory skills

1.4.1. **State principles and procedures of washing, sterilisation and care of glassware and other equipment in accordance with laboratory manuals**

1.4.2. **Make up solutions accurately from written formulae, including use of electronic balance and methods for adjustment of pH**

1.4.3. **Demonstrate the use and maintenance of microscopes including inverted, standard and fluorescent microscopes**

1.4.4. **Aliquot, dilute and store appropriately reagents for use in the laboratory**

1.4.5. **Explain the calibration, operation and maintenance of the equipment used in the performance of immunological assays**

1.5. Collection, receipt, storage, management and processing of specimens

1.5.1. Sample collection

1.5.1.1. Perform venesection

1.5.2. Specimen reception

1.5.2.1. State the principles, with reference to the laboratory procedures manual, of assessment of specimens into the laboratory, considering:

1.5.2.1.1. Nature of the sample

1.5.2.1.2. Collection conditions of the sample

1.5.2.1.3. Transport of the sample

1.5.2.1.4. Criteria for acceptance or rejection of a sample for processing

1.5.2.1.5. Storage of the sample, especially temperature considerations (RT, -4°C, -20°C, -70°C, liquid nitrogen)

1.5.2.1.6. Retention of the sample

1.5.2.1.7. Disposal of the sample

1.5.2.1.8. Patient origin and identity

1.5.2.2. State regulatory requirements for valid test ordering

1.5.2.3. State principles underlying the appropriate processing of samples dependent on their time of arrival and priority, considering working hours and urgency

1.5.3. Preparation of samples

1.5.3.1. Explain the procedure and be able to perform separation of samples for laboratory analysis

1.5.3.1.1. Serum separation

1.5.3.1.2. Concentration of body fluids (urine, CSF)

1.5.3.1.3. Ficoll-hypaque separation of peripheral blood lymphocytes

1.5.3.1.4. Preparation of a cell suspension from solid tissue

- 1.5.3.1.5. Separation of neutrophils from whole blood
- 1.5.3.2. Be familiar with advanced cell purification techniques
 - 1.5.3.2.1. Magnetic bead separation
 - 1.5.3.2.2. Flow cytometry-based sorting
 - 1.5.3.2.3. Percoll gradient density centrifugation
- 1.5.3.3. Explain the procedure and be able to perform preparation of a cell suspension at a given cellular density
 - 1.5.3.3.1. Count cells in a haemocytometer
 - 1.5.3.3.2. Determine the viability of a cell suspension using dye exclusion
- 1.5.3.4. Explain the procedure for storing viable cells including recovery
- 1.5.3.5. Maintain cell lines in culture
- 1.5.3.6. Explain in general terms the various molecular techniques, including:
 - 1.5.3.6.1. DNA and RNA extraction
 - 1.5.3.6.2. Polymerase chain reaction
 - 1.5.3.6.3. Hybridisation assays
 - 1.5.3.6.4. Sequencing

1.6. Validation, recording and reporting of laboratory data

- 1.6.1. **Demonstrates ability to use laboratory information systems to**
 - 1.6.1.1. Log in a specimen
 - 1.6.1.2. Enter results
 - 1.6.1.3. Verify a result or series of results
 - 1.6.1.4. Retrieve results

1.7. Provide clinical liaison in the clinical interpretation of diagnostic immunopathology test results

- 1.7.1. **Explain and justify an appropriate approach to the communication of abnormal test results to the ordering clinician**
 - 1.7.1.1. Define 'action limits'
- 1.7.2. **Provide interpretative information on all immunopathology testing within the clinical context**
 - 1.7.2.1. Develop and record an expert opinion, on the basis of a combination of clinical presentation and laboratory results, as to the nature, causation, severity and likely sequelae of diseases relevant to immunopathology testing
 - 1.7.2.2. Construct written reports containing all appropriate interpretative information, including pertinent recommendations to the requesting clinician
 - 1.7.2.3. Communicate such reports and opinions to referring clinicians verbally (in English) in a clear, concise and unambiguous fashion
 - 1.7.2.4. Contribute an expert opinion on immunopathology tests and their interpretation at clinical meetings, such as Grand Rounds, clinico-pathological conferences, morbidity and mortality reviews, quality and audit committees
 - 1.7.2.5. Seek further expert opinion where appropriate

2. Functions as a manager in the laboratory

2.1 Quality control and assurance

- 2.1.1. **Explain and apply the use of calibrators and controls in immunoassay**
 - 2.1.1.1. Standardisation
 - 2.1.1.2. Traceability of calibrators
 - 2.1.1.3. Choice of control samples
 - 2.1.1.4. Criteria for assay run acceptance/rejection, including Westgard rules
 - 2.1.1.5. Inter-assay and intra-assay variation
 - 2.1.1.6. Co-efficient of variation
 - 2.1.1.7. Random versus systematic errors
 - 2.1.1.8. Bias
- 2.1.2. **Explain the contribution of interference in immunoassays, including methods for detection and minimisation**
 - 📖 *Kricka LJ. Human anti-animal antibody interferences in immunological assays. Clinical Chemistry. 45(7):942-56, 1999 July*
 - 2.1.2.1. Heterophile antibodies
 - 2.1.2.2. RFs
 - 2.1.2.3. Cryoglobulins
- 2.1.3. **QC in flow cytometry**
 - 2.1.3.1. Outline strategies to monitor flow cytometer performance
 - 2.1.3.2. Explain methods for assuring quality control in quantification of lymphocyte subsets eg fixed cell suspensions
 - 2.1.3.3. Discuss QC of antibody reagents
- 2.1.4. **Quality Assurance Programs**
 - 2.1.4.1. Interpret external quality assurance (QA) reports, particularly the format used in Australian QAP programs
 - 2.1.4.2. Discuss sources of assay result variation in different laboratories

2.2. Trouble-shooting

- 2.2.1. **Recognise and rectify causes of error in immunopathology assays**

2.3. Selection of methodology

- 2.3.1. **Selects appropriate test methods and instruments taking into account factors such as:**
 - 2.3.1.1. Potential for automation
 - 2.3.1.2. Performance characteristics of test methods
 - 2.3.1.3. Quality control
 - 2.3.1.4. Calibration
 - 2.3.1.5. Training
 - 2.3.1.6. Reagent usage
 - 2.3.1.7. Waste disposal
 - 2.3.1.8. Costs and remuneration
 - 2.3.1.9. Service issues
 - 2.3.1.10. Maintenance
 - 2.3.1.11. Record keeping
 - 2.3.1.12. Ability to compare performance of different methods
 - 2.3.1.13. Statistical analysis
 - 2.3.1.14. Ability to assess test usage and clinical utility

2.4 Application of statistics to diagnostic testing

- 2.4.1. **Assessing assay performance**
 - 2.4.1.1. Define and explain terminology used in assay assessment and QC, including
 - 2.4.1.1.1. Precision

- 2.4.1.1.2. Accuracy
- 2.4.1.1.3. Inter-assay variability
- 2.4.1.1.4. Intra-assay variability
- 2.4.1.1.5. Selectivity, and influence of 'matrix' in immunoassays
- 2.4.1.2. Explain tools used to represent assay performance, including
 - 2.4.1.2.1. Levy-Jennings
 - 2.4.1.2.2. Cusum
- 2.4.1.3. Interpret internal quality assurance reports
 - 2.4.1.3.1. List, explain and apply the Westgard rules
- 2.4.1.4. Explain tools used to compare the performance of two assays, including
 - 2.4.1.4.1. Linear regression
 - 2.4.1.4.2. Bland-Altman plots
 - 2.4.1.4.3. Kappa statistic
 - 2.4.1.4.4. Chi-squared analyses
- 2.4.1.5. Explain the requirements to demonstrate the validity of an assay (full, partial, cross validation), including the measurement of
 - 📖 *Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Science International. 165(2-3):216-24, 2007 Jan 17.*
 - 2.4.1.5.1. Selectivity
 - 2.4.1.5.2. Precision
 - 2.4.1.5.3. Accuracy
 - 2.4.1.5.4. Recovery
 - 2.4.1.5.5. Lower limit of quantification
 - 2.4.1.5.6. Stability of reagents
 - 2.4.1.5.7. Interfering substances
- 2.4.1.6. Compare various assay methods and discuss factors determining the selection of an optimal methodology
- 2.4.2. **Reference ranges (RRs)**
 - 2.4.2.1. Define and explain standard statistical terminology, including
 - 2.4.2.1.1. Mean
 - 2.4.2.1.2. Median
 - 2.4.2.1.3. Standard deviation
 - 2.4.2.1.4. Co-efficient of variation (CV)
 - 2.4.2.1.5. Normal (Gaussian) distribution
 - 2.4.2.1.5.1. Tests for normality
 - 2.4.2.1.5.2. Outliers
 - 2.4.2.1.6. 95% confidence intervals
 - 2.4.2.1.6.1. Parametric
 - 2.4.2.1.6.2. Non-parametric
 - 2.4.2.2. Explain the process of establishing a RR, considering factors including
 - 2.4.2.2.1. Defining normal (disease versus health)
 - 2.4.2.2.2. Age
 - 2.4.2.2.3. Sex
 - 2.4.2.2.4. Ethnicity
 - 2.4.2.3. Explain the process of transferring RRs
- 2.4.3. **Measurement of Uncertainty (MOU)**
 - 2.4.3.1. Explain the principles behind the MOU
 - 2.4.3.2. Determination the MOU in appropriate assays
 - 2.4.3.3. Explain the clinical relevance of MOU in specific assays with regard to patient monitoring
- 2.4.4. **Diagnostic application of assay results**
 - 2.4.4.1. Construct a two-by-two table and derive implications for assessing diagnostic tests

- 2.4.4.1.1. Define and explain sensitivity, specificity, positive predictive value, negative predictive value
- 2.4.4.1.2. Define and explain the concept of odds ratio
- 2.4.4.2. Explain the principles of Receiver Operating Characteristics (ROC) curves and their application to assay cut-offs
 - 2.4.4.2.1. Area under ROC curve analysis
- 2.4.4.3. Explain Bayes theorem
 - 2.4.4.3.1. Pre-test and post-test probability
 - 2.4.4.3.2. Likelihood ratios

2.5 Laboratory safety

- 2.5.1. **States the employer's obligations under the OH&S act**
- 2.5.2. **Explain the concepts of risk management in the laboratory, including**
 - 2.5.2.1. Identification of hazards
 - 2.5.2.2. Assessment of risk
 - 2.5.2.3. Risk control
- 2.5.3. **Explain basic safety principles in the laboratory with regard to universal precautions, including**
 - 2.5.3.1. Appropriate attire
 - 2.5.3.2. Protective clothing
 - 2.5.3.3. Gloves
 - 2.5.3.4. Consumption of food
 - 2.5.3.5. Waste disposal
- 2.5.4. **Explain specific aspects of maintaining a safe working environment, including**
 - 2.5.4.1. Radioactivity
 - 2.5.4.2. Disinfection
 - 2.5.4.3. Chemical safety, including MSDSs
 - 2.5.4.4. Biosafety hoods
 - 2.5.4.5. Eye protection
 - 2.5.4.6. Fire procedures
 - 2.5.4.7. Lasers
 - 2.5.4.8. Liquid nitrogen
 - 2.5.4.9. Electrical equipment
 - 2.5.4.10. Carcinogens
- 2.5.5. **Explain basic aspects of ergonomics in the workplace**

2.6 Compliance with regulatory requirements and legislation

- 2.6.1. **States the principles of NATA ISO15189**
- 2.6.2. **States the regulatory requirements for retention of results and storage of samples**
- 2.6.3. **Explains the roles of critical regulatory bodies in Australian Pathology, including**
 - 2.6.3.1. RCPA
 - 2.6.3.2. NATA
 - 2.6.3.3. TGA
 - 2.6.3.4. NPAAC
 - 2.6.3.5. PSTC

2.7 Managing people

- 2.7.1. **Participate in discussion of organising laboratory staff, including**
 - 2.7.1.1. Rosters
 - 2.7.1.2. Training, including training manuals and assessment of competence
 - 2.7.1.3. Continuing education

2.8 Managing resources

- 2.8.1. **Explain factors which determine the cost of a test, considering**
 - 2.8.1.1. Fixed costs

- 2.8.1.1.1. Equipment
- 2.8.1.1.2. Service contracts
- 2.8.1.1.3. Administration
- 2.8.1.2. Variable costs
- 2.8.1.3. Direct costs (of assay)
 - 2.8.1.3.1. Reagents
 - 2.8.1.3.2. Labour
 - 2.8.1.3.3. Disposables
- 2.8.1.4. Indirect costs (infrastructure)
- 2.8.1.5. WELCAN unit
- 2.8.2. **Budget planning**
 - 2.8.2.1. Participate in discussion of budget issues pertaining to the management of the laboratory